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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 9/08, 9/19, 47/10, 47/18		A2	(11) International Publication Number: WO 99/39694
			(43) International Publication Date: 12 August 1999 (12.08.99)
(21) International Application Number: PCT/EP99/00702			(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 3 February 1999 (03.02.99)			
(30) Priority Data: 9802451.6 5 February 1998 (05.02.98) GB 9813646.8 24 June 1998 (24.06.98) GB			
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(54) Title: COMPOSITIONS CONTAINING ORGANIC COMPOUNDS			
(57) Abstract Pharmaceutical formulations comprising en epothilone in the form of an infusion concentrate or a lyophilised composition, and methods of administration of an epothilone in suitable form for parenteral administration.			

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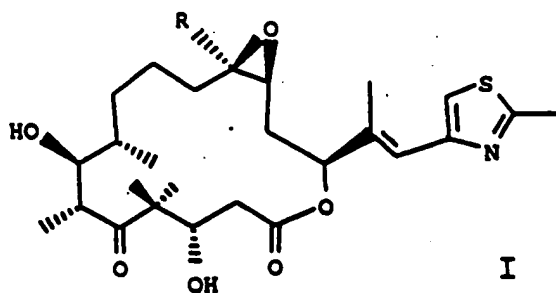
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COMPOSITIONS CONTAINING ORGANIC COMPOUNDS

This invention is concerned with pharmaceutical formulations of epothilones, and in particular pharmaceutical formulations which are administrable parenterally, e.g. intravenously.

The epothilones represent a class of microtubule stabilizing cytotoxic agents (see Gerth, K. et al., J. Antibiot. 49, 560-3 (1966); or Hoeffle et al., DE 41 38 042) of the formula I. Typical representatives include epothilone A wherein R is a hydrogen and epothilone B wherein R is a methyl group.



They are 16-member macrolides containing seven chiral centers and may also be characterized by various functionalities. For example, they may include other ring systems, such as an epoxide and/or a thiazole ring. They may have two free, derivatizable hydroxyl groups and the macrolide itself may comprise an ester linkage. The epothilones and their syntheses are described for example in published PCT application number WO 93/10121 and DE 41 38 042 A2, the contents of which are incorporated herein by reference. Typical epothilone derivatives and their syntheses are described in published PCT application number WO 97/19086 and WO 98/25929, the contents of which are incorporated herein by reference. Reference to the epothilones is preferably intended to mean epothilone A or epothilone B or their salts and derivatives or mixtures thereof as appropriate. Epothilone A or B may be used alone or they may be used as mixtures of A and B, preferably however they are used as solely A or solely B, most preferably solely B.

Cytotoxic agents are well known for the treatment of tumours. The anti-tumour activity of many of these compounds relies on the inhibition of cell proliferation and consequent induction of apoptosis and cell death. The majority of cytotoxic agents exert their effects

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through interference of DNA and/or RNA syntheses. However, for certain cytotoxic agents, e.g. members of the taxane family, e.g. paclitaxel, and the epothilones, their activity is reliant on their interference with microtubule dynamics. Microtubules are an important and attractive target for development of novel anti-cancer formulations.

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However, little has been published on formulations suitable for epothilones. We have found that the 16-member macrolide system is particularly labile to degradation. Moreover, the poor solubility of these compounds makes it very difficult to form pharmaceutical formulations for parenteral administration. Poorly soluble compounds conventionally may be brought into solution by warming the solvent during the dissolution process. However, given the high reactivity of these compounds they may be prone to degradation at elevated temperatures. Further, these highly reactive compounds may degrade over prolonged periods of storage as aqueous solutions. Concentrated solutions of the microtubule agent Taxol[®] which can be diluted in an aqueous medium prior to intravenous administration have been described. However, such solutions conventionally employ a surfactant such as Cremophor[®] (polyethoxylated castor oil). It is well known that surfactants such as Cremophor[®] can cause allergic reactions in patients.

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Thus there is a need for commercially acceptable pharmaceutical formulations suitable for epothilones, e.g. pharmaceutical formulations which allow for storage, e.g. in a refrigerator, e.g. at 2-8 °C.

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We have now surprisingly found means to improve the solubility of an epothilone, e.g. epothilone A or epothilone B, and/or render them more rapidly soluble without the use of a surfactant, for example a surfactant having an HLB value of 10 or more, e.g. Cremophor[®], and without adversely affecting their potency.

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Accordingly, the invention provides in one of its aspects a pharmaceutical formulation comprising an epothilone, e.g. epothilone A or epothilone B, which hereinafter may be referred to as a formulation of the present invention.

In a preferred embodiment the invention provides a pharmaceutical formulation in the form of an infusion concentrate which comprises an epothilone and a pharmaceutically acceptable organic solvent, e.g. in the absence of a surfactant having an HLB value of 10

- or above, e.g. Cremophor[®]. The infusion concentrate does not require the use of a surfactant to improve the solubility of an epothilone, e.g. epothilone A and B, and/or to render it more rapidly soluble. As stated above, surfactants such as a polyhydrogenated natural or hydrogenated castor oil, e.g. of an HLB value greater than 10, e.g. Cremophor[®], may cause allergic reactions and they also can leach plasticisers from standard PVC containers, tubing and the like. Consequently, when they are employed one may be required to use special infusion apparatus, e.g. nitro-glycerine tubing and non-plasticised containers, such as glass, tubing and the like.
- 10 The aforementioned pharmaceutically acceptable organic solvent may be chosen from any such organic solvent known in the art. Said solvent may be used individually or as combinations with other solvents. Preferably said solvent is liquid at room temperature. Preferably the solvent is selected (i) from an alcohol with a carbon chain length of at least 2, e.g. C₂-C₆, e.g. C₂ or C₃ or C₄, or (ii) from an N-alkylpyrrolidone, e.g. C₁-C₄, e.g. N-
- 15 methylpyrrolidone. Typical examples of alcohols are, e.g. a water miscible alcohol, e.g. absolute ethanol, or glycerol. Other alcohols include glycols, e.g. any glycol obtainable from an oxide such as ethylene oxide, e.g. propylene glycol. Other examples are polyols, e.g. a polyalkylene glycol, e.g. poly(C₂₋₃)alkylene glycol. A typical example is a polyethylene glycol, e.g. of a preferred molecular weight of 200-600 daltons, more preferably, 200-400
- 20 daltons, especially 300 daltons. Polyethylene glycols may be used in distilled form and may be characterised for example by one or more of the following features: (i) an ethylene oxide content of maximally 20 ppm, typically less than 1 ppm, e.g. 0.1 to 0.5 ppm, (ii) a pH value between 4.0 to 7.0, and (iii) the absence of reducing substances and aldehydes (as determined by examination of the degree of coloration of the liquid to be examined in
- 25 comparison with a reference solution comprising ferric chloride salts (yellow solution) or cobalt chlorides salts (red solutions), according to experimental procedures described in European Pharmacopoeia, Third Edition, 1997, Council of Europe, Strasbourg, Chapter 2.2.2. Degree of coloration of liquids, p. 15 - 17, incorporated herein by reference. One skilled in the art would realize that polyethylene glycols of various molecular weights
- 30 may be used as long as they are physiologically acceptable. The aforementioned solvents may of course contain residual water arising out of their production or being taken up from the atmosphere, e.g. up to saturation, e.g. up to 2 %, e.g. up to 0.5 %, e.g. typically less than 0.1 %, e.g. from 0.01 to 0.05 %. If desired, the pharmaceutically acceptable solvent may be mixed with water ("added water"), e.g. about up to 45 % water, e.g. up to 30 %, e.g.

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20 %, e.g. 5 %. Typical examples include ethanol/water mixtures, e.g. 70% ethanol weight/volume (w/v), or polyethylene glycol/water mixtures, e.g. 90 % polyethylene glycol w/v.

- 5 The epothilones, for example epothilone A or epothilone B, may be present in an infusion concentrate in a concentration of 0.1 to 100 mg/ml, e.g. 1 to 100 mg/ml, more preferably 0.5 to 50 mg/ml, more preferably 0.5 to 10 mg/ml, most preferably 1 mg/ml.

- 10 An epothilone, e.g. epothilone A or epothilone B, may be used individually or as a mixture of epothilones, e.g. a mixture of epothilone A and B. Given the stronger anti-tumour activity of epothilone B it may be employed in a lower concentration than epothilone A in the formulation. When used alone it is preferable to employ a concentration of epothilone A of 0.1 to 100 mg/ml, e.g. 10 to 100 mg/ml, preferably 0.1 to 50 mg/ml, e.g. 20 to 50 mg/ml, and especially 1 mg/ml. Epothilone B if used alone, is preferably employed in a concentration of 15 0.1 to 50 mg/ml, e.g. 10 to 50 mg/ml, e.g. 1 to 50 mg/ml, and especially 1 mg/ml.

- A pharmaceutical formulation of the present invention in the form of an infusion concentrate may be produced by working up, e.g. dissolving, an epothilone in a pharmaceutically acceptable solvent of the invention, optionally with other excipients. Preferably, no other 20 excipients are present. If they are present they are present preferably in an amount of less than 5 %, e.g. less than 2 %, e.g. between 0.1 to 1.5 %.

- Infusion concentrates of the present invention are conveniently stored in suitable containers, e.g. vials, double-chamber vial systems, or ampoules. Typically the vials or 25 ampoules are made from glass, e.g. borosilicate or soda-lime glass. The vials or ampoules may be of any volume conventional in the art, preferably they are of a size sufficient to accommodate 1 to 5 ml, more preferably 2 ml, of an infusion concentrate. The containers may accommodate preferably a stopper that can be pierced, e.g. a sterile rubber stopper, which may provide an appropriate hermetic seal with the container to allow for transfer of a 30 liquid from or to the container.

The infusion concentrates of the present invention may be stable for an extended period of time, e.g. up to 12 to 36, .g. 24 months at temperatures of at least 2 to 8 °C, as indicated in standard stability tests, e.g. as described in the examples.

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Furthermore, the infusion concentrates exhibit little evaporation, they may be produced using conventional equipment, e.g. no explosion-proof equipment is necessary, and they can tolerate rubber stoppers when stored in containers, e.g. without causing the stoppers to degrade.

Infusion concentrates may be diluted in a pharmaceutically acceptable organic solvent, e.g. an aqueous medium, suitable for intravenous administration to form an infusion solution, before the epothilone is administered parenterally, e.g. intravenously, to a patient. It is understood that, parenteral administration includes administration by infusion or injection.

Accordingly, the invention provides in another of its aspects an infusion solution comprising in admixture an infusion concentrate as hereinabove defined and a diluent selected from a pharmaceutically acceptable organic solvent, which is preferably an aqueous medium.

The pharmaceutically acceptable organic solvent used as a diluent may be any of those solvents or combinations of solvents used in the infusion concentrate. Preferably however it consists of water, i.e. water-for-injection. The infusion solution preferably has the same or essentially the same osmotic pressure as body fluid. Accordingly, the diluent preferably contains an isotonic agent or agents which has the effect of rendering the osmotic pressure of the infusion solution the same or essentially the same as body fluid.

The isotonic agent or agents may be selected from any of those known in the art, e.g. mannitol, dextrose, glucose and sodium chloride. Preferably the isotonic agent is glucose or sodium chloride. The isotonic agent or agents may be used in amounts which impart to the infusion solution the same or essentially the same osmotic pressure as body fluid. The precise quantities needed can be determined by routine experimentation and may depend upon the composition of the infusion solution and the nature of the isotonic agent or agents. Selection of a particular isotonic agent or agents may be made having regard to the properties of the epothilone, e.g. epothilone A or epothilone B. For example, when epothilone B is employed alone or in combination with epothilone A, the use of certain isotonic agent or agents may cause the infusion solution to turn turbid. The turbidity may be attributed to the dissolution of the epothilone, e.g. epothilone B.

Surprisingly, we have found that if one employs glucose as the isotonic agent then the turbidity does not appear for long periods, e.g. exceeding 24 hours, if at all.

- 5 The concentration of isotonic agent or agents in the aqueous medium will depend upon the nature of the particular isotonic agent or agents used, preferably the concentration is 5 % or less. When glucose is used it is preferably used in a concentration of from 1 to 5% w/v, more particularly 5% w/v. When the isotonic agent is sodium chloride it is preferably employed in amounts of up to 1% w/v, in particular 0.9% w/v.

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Infusion solutions according to the invention may comprise other excipients commonly employed in formulations to be administered intravenously. Excipients include antioxidants. Antioxidants may be employed to protect the epothilone, e.g. epothilone B, against oxidative degradation. Antioxidants may be chosen from any of those antioxidants known in
15 the art and suitable for intravenous formulations. The amount of antioxidant may be determined by routine experimentation. As an alternative to the addition of an antioxidant, or in addition thereto, the antioxidant effect may be achieved by displacing oxygen (air) from contact with the infusion solution. This may be conveniently carried out by purging the container holding said infusion solution with an inert gas, e.g. nitrogen.

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- The amount of diluent used in admixture with the infusion concentrate in order to form an infusion solution may be chosen according to the desired concentration of epothilone, e.g. epothilone B, in the infusion solution. Preferably the infusion solution is prepared by mixing a vial or ampoule of infusion concentrate aforementioned with a diluent, e.g. a 5 % w/v
25 glucose solution in water-for-injection in a suitable container, e.g. an infusion bag or bottle, making the volume up to between 50 ml and 1000 ml, e.g. 200 ml and 1000 ml or preferably 50 to 100 ml, with the diluent. The infusion solution so formed may be preferably used immediately or within a short time of being formed, e.g. within 6 hours. Alternatively, the infusion concentrate and a predetermined amount of diluent, may be loaded each into
30 separate chambers of a double-chamber vial system and only mixed immediately prior to intravenous administration to a patient.

In an alternative embodiment a pharmaceutical formulation of the present invention may be in the form of a lyophilised composition comprising an epothilone, e.g. epothilone A or

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epothilone B. Given the poor solubility of epothilone A and B a lyophilisate mass consisting only of an epothilone, e.g. epothilone A or epothilone B, may be very small such that it does not provide a lyophilised composition of suitable bulk to be handled conveniently, e.g. giving acceptable content uniformity, or even to the extent that it may even be difficult to detect visually. Accordingly, one may use excipients in a lyophilised composition according to the invention which act to increase the solids content and therefore the bulk of the lyophilised composition. Suitable excipients may be any of those excipients which used alone or in combination will increase the bulk of the lyophilised composition without adversely interacting with the epothilone such as to destabilise the epothilone or otherwise reduce its potency. Additionally, the excipients need to be suitable for use in pharmaceutical formulations, e.g. parenteral, e.g. intravenous pharmaceutical formulations. Therefore when selecting an excipient or excipients consideration must be given not only to the nature of the lyophilised composition but also to the nature of the final pharmaceutical form. Examples of suitable excipients include sodium or potassium phosphates, citric acid, tartaric acid, gelatin, lactose and other carbohydrates such as dextrose, mannitol and dextran and any of the cyclodextrins which are suitable for use intravenously, e.g. a beta-cyclodextrin. Typical beta-cyclodextrins include also beta-cyclodextrin derivatives, e.g. alkyl- or allyl-derivatives, or hydroxyalkyl-derivatives, which are for example formed by a condensation reaction of beta-cyclodextrin with an oxide, e.g. propyleneoxide. In a more preferred embodiment the beta-cyclodextrin derivative may be hydroxypropyl-beta-cyclodextrin. Preferably the hydroxypropyl-beta-cyclodextrin may be any of those mentioned by Roger A. Rajewski et al in the Journal of Pharmaceutical Sciences, Vol. 85, No. 11, November 1996, pages 1142 through 1169, which article is incorporated herein by reference.

Through judicious selection of excipients the applicant has found that a suitably bulky lyophilised composition comprising an epothilone, e.g. epothilone A or epothilone B, may be formed which exhibits improved solubility characteristics of an epothilone, e.g. epothilone A or epothilone B, or renders the epothilone more rapidly soluble but which does not adversely affect the potency of the epothilone.

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The excipients or mixtures thereof may contribute to 50 to 99.9 % of the total solids content of the lyophilised composition, more preferably 90 to 99 %, e.g. 95 % of total solids of said composition.

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The epothilone may contribute 100% to the total solids content of the lyophilised composition although preferably it may contribute to 0.1 to 10 %, more preferably 0.1 to 1.5 %, e.g. 1.2% of total solids.

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To the extent that the epothilone and cyclodextrin or mannitol do not provide 100% of the total solids content of the lyophilised composition, the balance of solids may be provided by any excipients commonly used in the field of lyophilisates which are to be reconstituted for pharmaceutical use, e.g any of the other excipients referred to hereinabove.

10

Lyophilised compositions according to the invention may be formed from solutions (hereinafter referred to as "original solutions") containing an epothilone, e.g. epothilone A or epothilone B, and suitable excipients as defined hereinabove. Suitable solvents for such original solutions are either water alone or aqueous based solvents containing

15 pharmaceutically acceptable, water miscible organic solvents, e.g. alcohols, more particularly ethanol or polyethylene glycol.

Original solutions may contain from 0.01% to 0.5% (w/v) of epothilone, e.g. epothilone A or epothilone B.

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Original solutions may be prepared by dissolving the epothilone, e.g. epothilone A or epothilone B, and excipients in a suitable solvent and thereafter filtering the solution through a filter, e.g. a sterile 0.22 micron filter. The original solution thus formed may be filled into vials of suitable volume, preferably having a volume of 30 ml and a fill volume of

25 4.2 ml.

In yet another aspect the present invention provides a method of producing a lyophilised composition which comprises the steps of (i) mixing an epothilone, e.g. epothilone A or epothilone B, with a pharmaceutically acceptable excipient, e.g. mannitol or a cyclodextrin, e.g. a hydroxypropyl-beta-cyclodextrin in a suitable solvent to form an original solution, and

30 (ii) dehydrating the original solution.

Lyophilisation may be carried out according to known techniques. In a preferred process the aforementioned filled vials may be frozen in a lyophilisation chamber for approximately 3

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hours at a temperature below the eutectic point, preferably approximately - 40 °C.

Thereafter the lyophilisation chamber may be evacuated to about 0.1 to 0.2 millitorr. The temperature of the chamber may then be increased to effect sublimation of the frozen liquids. Preferably the temperature is increased to about 0 °C and this temperature may be maintained for a period of 8 to 15 hours to effect lyophilisation.

The lyophilised composition may be used in the production of parenteral formulations and so the lyophilisation process is preferably carried out under sterile conditions, for example using aseptic production processes or by irradiation. Aseptic formation of solutions containing pharmaceutically active compounds, the aseptic filling of vials and lyophilisation processes under aseptic conditions are well known to the skilled addressee.

The moisture content of the lyophilised composition thus formed may be 3% or less of the total weight of the lyophilised composition. Optionally however, a humidification step may be employed subsequent to lyophilisation wherein sterile water vapour may be introduced into the lyophilisation chamber at atmospheric pressure or at a reduced pressure as aforementioned. Of course, if the humidification step is carried out under reduced pressure, the pressure may vary with the introduction of the water vapour, and pressure changes can be monitored and pressure adjusted if necessary using techniques well known in the art.

The humidification step may be completed with a time period of from 4 to 8 hours depending on whether it is carried out at atmospheric pressure or reduced pressure.

Lyophilised compositions obtained using a humidification step are hereinafter referred to as hydrated lyophilisates. Said hydrated lyophilisates may contain from 0.1 to 5 % by weight of water.

Lyophilised compositions according to the present invention may be provided in single dosage container forms. The single dosage container forms may be of any suitable size. By "suitable size" is meant an appropriate size having regard to the volume of solution which will be needed to reconstitute the lyophilised composition. Any suitable containers may be used to provide these dosage forms. By "suitable" is meant any container which may be used in aseptic filling procedures and which is capable of maintaining a sterile environment and which is unreactive to the lyophilised composition. Preferred containers may be formed of glass, e.g. Type I glass and may have means to receive a stopper, e.g. a sterile rubber

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stopper which may cooperate with the walls of the container to provide a hermitic seal. Preferred stoppers also may allow entry to the contents of the container for the purpose of introduction of a solvent, e.g. water for injection, for the lyophilised composition.

- 5 The lyophilised composition according to the invention may be storage stable for up to 24 to 36 months at a temperature of 2 to 30 °C. Lyophilised compositions stored for these periods display no signs of degradation and the solubility characteristics remain unaffected.

- 10 When it is desired to provide a parenteral, e.g. intravenous, form of an epothilone, the lyophilised composition may be re-constituted, preferably just before administration.

- Re-constitution may involve dissolving the lyophilised composition in water or some other pharmaceutically acceptable solvent as hereinabove described, for example physiological saline, an aqueous solution of a pharmaceutically acceptable alcohol, e.g. ethanol, propylene glycol, a polyethylene glycol, e.g. polyethylene glycol 300, and the like, or some other sterile injectable under aseptic conditions. The single dosage container form may be filled with an appropriate quantity of solvent having regard to the desired concentration of epothilone, e.g. epothilone A or epothilone B, required for parenteral administration. Such a reconstituted lyophilised composition may be preferably used immediately or within a short time of being formed, e.g. within 6 hours.
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- A pharmaceutical formulation of the present invention in suitable form for parenteral, e.g. intravenous administration, e.g. an infusion solution prepared by diluting an infusion concentrate or a reconstituted lyophilised composition (hereinafter referred to as diluted formulations of the present invention), may be filled in containers chosen from any conventional container which is non-reactive to said pharmaceutical formulations. Glass containers made from those glass types aforementioned are suitable although it is preferred to use plastics containers, e.g. plastic infusion bags.
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- 30 Plastics containers may be principally those composed of thermoplastic polymers. Plastics materials may additionally comprise additives, e.g. plasticisers, fillers, antioxidants, antistatics and other additives conventional in the art.

Plastics suitable for diluted formulations of the present invention should be resistant to elevated temperatures required for thermal sterilisation. Preferred plastics infusion bags are those made from PVC plastics materials known in the art.

- 5 A wide range of container sizes may be employed. When selecting a container size, consideration may be paid to the solubility of the epothilone in the particular solvent and the ease of handling and, if appropriate, storage of the container. It is preferred to use containers which can accommodate between about 200 to 1000 ml, e.g. 250 to 1000 ml, of a diluted formulation of the present invention.

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A diluted formulation of the present invention may be preferably sterile. This may be readily accomplished, e.g. by irradiation or by filtration of said diluted formulation through sterile filtration membranes. Aseptic formation of any composition in liquid form, the aseptic filling vials and/or combining of liquids for parenteral use with a suitable diluent under aseptic conditions are well known to the skilled addressee.

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- A diluted formulation of the present invention is useful for treatment and prevention of malignant proliferative disorders, for example the indications and conditions disclosed in WO 93/10121 and DE 41 38 042 A2, the contents of which are incorporated herein by reference. More specifically, they may be useful for the treatment of a tumour disease, e.g. a melanoma, ovarian cancer, pancreas cancer, neuroblastoma, head and neck cancer, bladder cancer, renal, brain, gastric or preferably a colorectal, prostate, breast, lung (especially non-small cell lung) or epithelial, especially epidermoid, e.g. cervical, cancer. Moreover, a diluted formulation of the present invention is beneficial in treating conditions for which and in the same manner as Paclitaxel[®] is used. For certain tumours epothilones offer enhanced beneficial effects compared with Paclitaxel[®]. For certain tumours, e.g. certain types of lung tumours, e.g. A549 lung epothilone B offers enhanced beneficial effects compared with Paclitaxel[®].

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- 30 Generally, a diluted formulation of the present invention may be administered in an amount which is therapeutically effective against a proliferative disease that can be treated by administration of an epothilone, e.g. epothilone A and/or epothilone B, especially epothilone B. Such proliferative diseases include any proliferative disease as mentioned above, especially a tumour disease, the response to a therapeutically effective amount preferably

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manifesting itself in a diminished proliferation, e.g. diminished tumour growth or even (more preferably) tumor regression or (most preferably) tumour disappearance. The exact amount and the duration of administration may depend upon the nature of the epothilone, e.g. epothilone A, epothilone B or a mixture of both, the particular type of malignantly

5 proliferating cells characteristic of the particular tumour, the seriousness of the condition, the rate of administration, as well as the patient's health and response to treatment.

Also, a pharmaceutical formulation of the present invention in suitable form for parenteral administration, e.g. an infusion solution prepared by diluting an infusion concentrate or a

10 reconstituted lyophilised composition, may be combined with other tumour treatments known to a skilled person, e.g. radiation, or administered as part of a combination therapy comprising at least one other chemotherapeutic agent. The administration of a combination of active agents may be simultaneous or consecutive, with either one of the active agents being administered first. The dosage of the active agents of a combination treatment may

15 depend on effectiveness and site of action of each active agent as well as synergistic effects between the agents used for combination therapy.

Other chemotherapeutic agents may include especially any chemotherapeutic agent that is or can be used in the treatment of tumor diseases, such as chemotherapeutics derived from

20 the following classes:

- (A) alkylating agents, preferably cross-linking chemotherapeutics, preferably bis-alkylating agents,
- (B) antitumour antibiotics, preferably doxorubicin (Adriamycin[®], Rubex[®]);
- (C) antimetabolites;
- 25 (D) plant alkaloids;
- (E) hormonal agents and antagonists;
- (F) biological response modifiers, preferably lymphokines or interferons;
- (G) inhibitors of protein tyrosine kinases and/or serine/threonine kinases;
- (H) antisense oligonucleotides or oligonucleotide derivatives; or
- 30 (I) miscellaneous agents or agents with other or unknown mechanism of action, preferably of the Taxane class, especially Taxotere[®] or most especially paclitaxel (Taxol[®]).

A diluted formulation of the present invention may, therefore, be useful as a single anti-cancer formulations or as part of a combination regimen for the treatment of various tumours.

- 5 The utility of all diluted formulations of the present invention may be observed in standard clinical trials in, for example, known indications of epothilone dosages giving equivalent blood levels of epothilone; for example using dosages in the range of about 0.1 to 6 mg/m² of epothilone for weekly treatment and about 0.3 to 18 mg/m² of epothilone for three-weekly treatment for a 75 kilogram mammal, e.g. an adult human of 1.73 m², and in standard
10 animal models. For example, the anti-tumor effect of single dose regimens are investigated in a model of human ovarian cancer SKOV3 as well as a U373 glioma model.

- The increased bioavailability of an epothilone administered in the form of a diluted formulation of the present invention, may be observed in standard animal tests and in
15 clinical trials, e.g. as described above. Naturally, the exact amounts of epothilone and of the pharmaceutical formulation to be administered may depend on a number of factors, e.g. the condition to be treated, the exact epothilone, the desired duration of treatment and the rate of administration of epothilone. For example, the amount of epothilone required and the administration rate thereof may be determined on the basis of known *in vivo* and *in vitro*
20 techniques, for example as described above, determining how long a particular epothilone concentration in the blood plasma remains at an acceptable level for a therapeutic effect.

- Diluted formulations of the present invention may be conveniently administered intravenously in a dosage of from about 0.1 to 100 mg/m², e.g. 0.2 to 100 mg/m² epothilone
25 A and from about 0.1 to 50 mg/m², e.g. 0.2 to 50 mg/m² of epothilone B. Preferably, for weekly treatment the dose is between 0.1 and 6 mg/m², preferably 0.1 and 5, more preferably 0.1 and 3, even more preferably 0.1 and 1.7 mg/m², most preferably 0.1 and 1 mg/m²; for three-weekly treatment the dose is between 0.3 and 18 mg/m², preferably 0.3 and 15, more preferably 0.3 and 12, even more preferably 0.3 and 7.5 mg/m², most
30 preferably 1.0 and 3.0 mg/m². This dose is preferably administered to a human by intravenous administration during 2 to 180 min, preferably 2 to 120 min, more preferably during 5 to 30 min, most preferably during 10 to 30 min, e.g. during 30 min.

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Preferably the concentration and dosage strength may be such to achieve an effective dose level of about 0.5 to 15 mg/day, more preferably 1 to 10 mg/day, more preferably 2 to 8 mg/day. The dose received by intravenous administration and the blood concentration may be determined accurately on the basis of known *in vivo* and *in vitro* techniques.

5

In yet another aspect the invention provides a method of administering an epothilone to a subject in need of epothilone treatment which comprises administering parenterally a diluted formulation of the present invention to a subject in need of such treatment. More specifically, such a method of administering an epothilone comprises (a) diluting a pharmaceutical formulation according to the invention, e.g. in the form of an infusion concentrate or a lyophilised composition, with an aqueous medium, to form a solution suitable for parenteral, e.g. intravenous, administration, and (b) administering such a solution to the subject.

10

15 In yet another aspect the invention provides use of an epothilone in the manufacture of a medicament suitable for parenteral administration.

20

The invention is illustrated by way of the following examples which are not intended to limit the scope of the present invention. All percentages are by weight/weight unless otherwise specified. Any components of the pharmaceutical formulations may further be described in Fiedler, H. P. "Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete", Editio Cantor, D-7960 Aulendorf, 4th revised and expanded edition (1996), the contents of which are hereby incorporated by reference.

EXAMPLES**Example 1**

Epothilone (15 mg of B or 50 mg of A) is dissolved in 98-100 % propylene glycol (1.0 ml).

- 5 The solution is sterile filtered through a 0.22 microns pore size filter and charged to 1 ml ampoules. The filled ampoules are used for storage and shipment. The filled ampoules are stable for a period of at least 12 months at a temperature of 2 to 8 °C. Prior to intravenous administration, the contents of an ampoule are added to 250 to 1000 ml of a 5 % glucose solution in water-for-injection. The intravenous solution thus formed is stable for a period of
- 10 8 hours at room temperature.

Examples 2 to 7

The experiment of Example 1 is repeated using the absolute and aqueous ethanol solvent systems and various polyethylene glycol solvent systems (Table 1).

15

Table 1:

Example	Solvent system
2	Absolute ethanol
3	Polyethylene glycol 300
4	Polyethylene glycol 400
5	50 to 100 % ethanol
6	Polyethylene glycol 300: 90 to 100 %
7	Polyethylene glycol 400: 90 to 100 %

- The infusion solutions obtained from Examples 2 to 7 are all stable for a period of 8 hours
- 20 at room temperature.

Example 8

Solubilities in various solvent systems are summarized in Table 2. If not indicated otherwise all solubility data refer to T = 22 °C.

25

Table 2: Solubility of epothilone B in various solvent systems.

Solvent/Solvent-system		Solubility [g/l] \pm 10%
H ₂ O (pH 6.0)		0.16 (0.19, 4 °C)
Phosphate buffer pH 7.4		0.16 (0.19, 4 °C)
H ₂ O, 0.9% glucose		0.16
H ₂ O, 5% glucose		0.16
H ₂ O, 15% glycerin		0.19
H ₂ O, 5% poloxamer 188		0.23
EtOH/H ₂ O (v/v)	100/0	> 50
	50/50	27
	30/70	4.1
	20/80	1.3
	10/90	1.1
PEG 300/H ₂ O (v/v)	100/0	12
	50/50	10
	70/30	2.5
PEG 400/H ₂ O (v/v)	100/0	30
	50/50	11
	70/30	2.3
Propylene glycol/H ₂ O (v/v)	100/0	26
	70/30	10.4
	50/50	1.6

- The solubility of epothilone B in water at neutral pH is about 160 mg/l, and significantly higher solubility is achieved in PEG/water, propyleneglycol/water, or EtOH/water mixtures. In comparison, previously reported aqueous solubility of epothilone A is 940 mg/l and 700 mg/l for mixtures of epothilones A and B.

Example 9

- The stability of aqueous versus nonaqueous polyethyleneglycol infusion concentrates comprising epothilone B at different concentration and various temperatures is determined. Typically, a known amount of epothilone B is dissolved in 1.0 ml of each of the various solvent systems and each solution is sterile filtered and charged to 1 ml white-glass vials with grey rubber stoppers and grey flip-off caps. Table 3 describes the amount of

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degradation product formed over a period of up to seven months. The stability is analyzed by determining formation of degradation products in each of the infusion concentrates as a function of time and temperature. Each sample is analyzed by HPLC, the sample is prepared by diluting the concentrate with an aqueous medium. The stability of all infusion concentrates after 3 months at 2 to 8 °C is comparable. At higher temperatures, e.g. 25 °C, nonaqueous solvent systems comprising PEG exhibit generally higher stability than aqueous solvent systems comprising PEG.

Table 3 Proportion of degradation products in aqueous vs. nonaqueous PEG-containing infusion concentrates.

Solvent system	Dosage	Time	2-8 °C	25°C
PEG 400	1 mg/ml	3 months	<0.1	0.2
PEG 400/ water 90:10 (w/w)	1 mg/ml	3 months	<0.1	0.4
PEG 300	1 mg/ml	3 months	<0.1	-
PEG 300/ water 90:10 (w/w)	15 mg/ml	7 months	<0.1	1.0
ethanol/water 59:41 (w/w)	5 mg/ml	initial 1 month 5 months	< 0.1 0.3 0.3	-

15 **Example 10**

An aqueous solution is prepared by dissolving epothilone B (5.0 mg) and mannitol (1500 mg) in water for injection to make up a 30 ml solution. The solution is passed through a sterile 0.22 micron pore size membrane filter before aseptically filling the solution into a glass vial and thereafter aseptically fitting a sterile stopper to the vial in readiness for the drying process. The filled vial is then positioned in a lyophilisation chamber and cooled to a temperature of about -40 °C. The lyophilisator condenser is cooled to about -60 °C and the chamber is evacuated to about 0.1 millitorr. The chamber temperature is set to about 20 °C to start the drying process. After about 20 hours of drying the chamber pressure has increased to about 0.2 millitorr and the drying process is deemed complete. The pressure of the chamber is increased to atmospheric pressure by aseptically introducing sterile air or nitrogen into the chamber. Thereafter the stopper is aseptically seated onto the vial to

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provide a hermitic sterile seal. The sealed vial provides a single dose container of epothilone B which is reconstituted shortly before administration with 25 ml of water for injection. The dosage is administered intravenously.

The lyophilised product possesses the desired characteristics required of the lyophilised compositions according to the invention.

Examples 11 to 14

The methodology of Example 10 is carried out in respect of the tabulated components set forth hereinbelow to form lyophilised products (Table 4).

Table 4

Example	Epothilone B mg	hydroxypropyl-beta cyclodextrin /mg	Water for Injection/ml
11	5.0	400	up to 4.2
12	5.0	500	up to 5.0
13	5.0	420	up to 4.2
14	5.0	420	up to 8.5

The lyophilised products formed according to Examples 11 to 14 possess the desired

characteristics required of the lyophilised compositions according to the invention.

CLAIMS

1. A pharmaceutical formulation in the form of an infusion concentrate comprising an epothilone and a pharmaceutically acceptable organic solvent.
- 5 2. A pharmaceutical formulation comprising an epothilone and a pharmaceutically acceptable organic solvent in the absence of a surfactant having an HLB value of 10 or above, e.g. Cremophor®.
- 10 3. A pharmaceutical formulation comprising an epothilone and a pharmaceutically acceptable organic solvent selected from (i) an alcohol or (ii) an N-alkylpyrrolidone.
4. A pharmaceutical formulation according to any of the preceding claims wherein the pharmaceutically acceptable organic solvent is a polyethylene glycol.
- 15 5. A pharmaceutical formulation according to any of the preceding claims additionally comprising added water.
6. A pharmaceutical formulation according to any of the preceding claims wherein water
- 20 is present in an amount of up to 45 % w/v.
7. A pharmaceutical formulation according to claim 7 wherein water is present in an amount of up to 0.5 % w/v.
- 25 8. A pharmaceutical formulation according to any of the preceding claims wherein the epothilone is at a concentration of 1 to 5 mg/ml.
9. An infusion solution comprising a formulation according to any of the preceding claims and a pharmaceutically acceptable solvent.
- 30 10. A lyophilised composition comprising (i) an epothilone and (ii) mannitol or a cyclodextrin.

- 20 -

11. A lyophilised composition comprising an epothilone and hydroxypropyl-beta-cyclodextrin.
- 5 12. A lyophilised composition according to claim 10 or 11 wherein an epothilone represents 0.1 to 1.5 % of the total solids.
- 10 13. A lyophilised composition according to claim 10 or 11 wherein an epothilone represents 0.1 to 1.5 % of the total solids and a cyclodextrin represents 90 to 99% of the total solids.
14. A reconstituted lyophilised composition comprising a pharmaceutical formulation as described in anyone of claims 10 to 13 in a pharmaceutically acceptable solvent.
- 15 15. A method of administering an epothilone which comprises :
 - (a) diluting a pharmaceutical formulation according to anyone of claims 1 to 8 with an aqueous medium to form an infusion solution and
 - (b) administering the infusion solution intravenously to the subject.
- 20 16. A method of administering an epothilone which comprises :
 - (a) reconstituting a lyophilised composition according to anyone of claims 10 to 13 with an aqueous medium to form a solution and
 - (b) administering the solution intravenously to the subject.
- 25 17. A method of administering an epothilone to a subject in need of epothilone treatment which comprises administering parenterally an infusion solution according to claim 9 to a subject in need of such treatment.
- 30 18. A method of administering an epothilone to a subject in need of epothilone treatment which comprises administering parenterally a reconstituted lyophilised composition according to claim 14 to a subject in need of such treatment.
19. Use of an epothilone in the manufacture of a medicament suitable for parenteral administration.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 9/08, 9/19, 47/10, 47/18, 31/335, 31/365		A3	(11) International Publication Number: WO 99/39694 (43) International Publication Date: 12 August 1999 (12.08.99)
(21) International Application Number: PCT/EP99/00702 (22) International Filing Date: 3 February 1999 (03.02.99) (30) Priority Data: 9802451.6 5 February 1998 (05.02.98) GB 9813646.8 24 June 1998 (24.06.98) GB (71) Applicant (for all designated States except AT US): NOVARTIS AG [CH/CH]; Schwarzwaldallee 215, CH-4058 Basel (CH). (71) Applicant (for AT only): NOVARTIS-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT MBH [AT/AT]; Brunner Strasse 59, A-1235 Vienna (AT). (72) Inventor; and (75) Inventor/Applicant (for US only): VAN HOOGEVEST, Peter [NL/CH]; Breitenstrasse 3, CH-4416 Bubendorf (CH). (74) Agent: BECKER, Konrad; Novartis AG, Corporate Intellectual Property, Patent and Trademark Dept., CH-4002 Basel (CH).			(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 23 September 1999 (23.09.99)

(54) Title: COMPOSITIONS CONTAINING ORGANIC COMPOUNDS

(57) Abstract

Pharmaceutical formulations comprising en epothilone in the form of an infusion concentrate or a lyophilised composition, and methods of administration of an epothilone in suitable form for parenteral administration.

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INTERNATIONAL SEARCH REPORT

Intern. Appl. No.

PCT/EP 99/00702

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K9/08 A61K9/19 A61K47/10 A61K47/18 A61K31/335
A61K31/365

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 10121 A (GESELLSCHAFT FÜR BIOTECHNOLOGISCHE FORSCHUNG) 27 May 1993 (1993-05-27) cited in the application claims 1,8	1-19
A	WO 97 19086 A (GESELLSCHAFT FÜR BIOTECHNOLOGISCHE FORSCHUNG) 29 May 1997 (1997-05-29) cited in the application claims 1,13	1-19
A	WO 97 33552 A (WALLACE TECHNOLOGIES INC., U.S.A.) 18 September 1997 (1997-09-18) claims 1,12,21,26 page 16, line 30 - page 17, line 27	1-19

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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3 August 1999

Date of mailing of the international search report

09/08/1999

Name and mailing address of the ISA

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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is(are) directed to a method of treatment of the human/animal
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Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9310121 A	27-05-1993	DE 4138042 A AU 2943792 A	27-05-1993 15-06-1993
WO 9719086 A	29-05-1997	DE 19542986 A DE 19639456 A EP 0873341 A EP 0903348 A	22-05-1997 26-03-1998 28-10-1998 24-03-1999
WO 9733552 A	18-09-1997	AU 2580697 A CA 2250295 A NO 984210 A PL 328807 A	01-10-1997 18-09-1997 11-11-1998 15-02-1999